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PRINCIPAL INVESTIGATOR: Dominique Broccoli, Ph.D.

CONTRACTING ORGANIZATION: Fox Chase Cancer Center
Philadelphia, PA 19111-2497

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Dominique Broccoli, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Fox Chase Cancer Center
Philadelphia, PA 19111-2497

E-Mail: K_broccoli@fccc.edu

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13. ABSTRACT (Maximum 200 Words)

The goal of this project is to elucidate some of the genetic and biological determinants of ovarian cancer, focusing on an *in vitro* model for ovarian cancer that we have developed. We found that immortalization and transformation of human ovarian surface epithelial (HOSE) cells can differ in the pathway used for telomere length maintenance, a phenomenon that we have also observed in the clinical disease. We have found that the majority of our HOSE cell cultures use the Alternative Lengthing of Telomeres (ALT) pathway for telomere maintenance, thereby providing an *in vitro* model to characterize the underlying basis of the ALT pathway in ovarian cancer. During this year of funding we have completed characterization of 50 ovarian tumors and found two that are telomerase negative and contain the characteristic long telomeres associated with the ALT pathway. We have also developed and optimized the technique to detect ALT-associated PML nuclear bodies in paraffin embedded tissues. This technology may distinguish additional ALT tumors among the cohort of telomerase negative tumors so far identified. Finally, we have improved our ability to profile RNA from these tumors by establishing and optimizing several technical aspects including isolation of RNA from microdissected tissue. The techniques developed in the last year will be critical in assessing expression patterns of ovarian tumors that use ALT for telomere maintenance.

14. SUBJECT TERMS

Alternative lengthening of telomeres (ALT), telomerase independent telomere maintenance, cDNA microarray analysis of differentially expressed genes, human ovarian surface epithelial (HOSE) cell, ovarian cancer

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Introduction

The diseases that are commonly referred to as ovarian cancer in the vast majority of cases develop from the malignant transformation of a single cell type, the surface epithelium. However, the biological mechanisms leading to transformation remain unclear. The goal of this project is to elucidate some of the genetic and biological determinants of ovarian cancer, focusing on an *in vitro* model for ovarian cancer that we have developed (1-4). We have initiated primary human ovarian surface epithelial (HOSE) cell cultures and have successfully derived HOSE cell lines that have undergone immortalization and spontaneous transformation *in vitro* and can form tumors *in vivo*. Furthermore, we found that immortalization and transformation of HOSE cells can differ in the pathway used for telomere length maintenance, a phenomenon that we have also observed in the clinical disease. Maintenance of telomeric repeats is required for immortalization and is commonly associated with activity of telomerase [reviewed in (5)]. However, a number of tumors and tumor cell lines have been described that do not have telomerase activity in which the telomere length dependent limitation on cell division is circumvented by a mechanism called Alternative Lengthening of Telomeres (ALT) (6). We have recently found that 30% of advanced stage ovarian adenocarcinomas lack telomerase activity and thus would be refractory to treatment with telomerase inhibitors. The ALT pathway also represents a salvage pathway that may be activated in tumors in response to short telomeres arising as a consequence of telomerase inhibition. We have found that the majority of our HOSE cell cultures use the ALT pathway for telomere maintenance, thereby providing an *in vitro* model to characterize the underlying basis of the ALT pathway in ovarian cancer. The mechanism(s) leading to ALT is unknown yet is clearly important in tumorigenesis. Using our HOSE tissue culture model and powerful molecular genetic screening tools we propose to uncover genes of relevance to ALT and malignant transformation of the ovarian surface epithelial cells.

Body

We have made progress on the following tasks proposed during the second year of funding:

Characterization of 50 ovarian tumors for mechanism of telomere maintenance

We have completed characterization of an additional 50 ovarian tumors for telomerase activity versus ALT (Figure 1 and Table 1). Of the 50 tumors characterized, 28 contained detectable telomerase activity using 0.5 ug of extract. The remaining tumor extracts did not exhibit telomerase activity. To confirm whether these tumors were in fact telomerase negative, additional TRAP assays using a range of extract (from 0.05 ug to 5 ug) were carried out. In addition, telomerase negative tumors were reassessed following preparation of a second extract. Upon completion of this analysis an additional 9 tumors were determined to contain telomerase activity. Therefore, 36 out of 50 ovarian tumors analyzed (72%) were found to be telomerase positive.

Southern blot analysis was carried out to determine telomere length in 9 of the 14 telomerase negative tumors where sufficient DNA was available. In addition, telomere length was determined for 16 of the telomerase positive tumors. This analysis yielded two tumors with the ultra-long telomeres (>20Kb) that are characteristic of the ALT pathway (Figure 2 and data not shown). The remaining 7 telomerase negative tumors had telomere lengths similar to those seen in telomerase positive tumors.

Cell lines that utilize the ALT pathway for telomere maintenance contain large multiprotein complexes in which telomeric proteins and DNA co-localize with the PML nuclear body, called ALT-associated PML nuclear bodies (APBs) (7). To determine if the tumors with telomere lengths within the size range typically seen of telomerase positive tumors were also using the ALT pathway for telomere maintenance, we are analyzing these tumors for the presence of APBs. This analysis required optimization of the ability to detect these structures in paraffin embedded tissue sections. This technical optimization has been carried out and we are able to detect APBs in paraffin embedded sections. Analysis of tumors is currently underway. In this aspect, we have recently improved our ability to

profile the RNA expression patterns of these tumors. First, we have derived oligonucleotides microarray. We have found that oligonucleotides microarrays give superior quality of hybridization signal relative to cDNA arrays by reducing non-specific binding and thus general background. These arrays contain 29,952 oligonucleotides that have been spotted onto polylysine coated microscopic glass slides. Each oligo in the set is 50 bp in length, correspond to HPSF® (High Purity Salt Free) quality standard and was designed to a coding region of a unique gene. Arrays have been spotted using a GeneMachine Omnigrid arrayer (GeneMachine, San Carlos, CA). From each batch of slides, one undergoes strict quality control for spot morphology and DNA abundance. Secondly, we have refined methods to amplify the RNA from microdissected tumor cells. We have recently evaluated RNA from ~10,000 microdissected cells. .

Key Research Accomplishments

- Completed characterization of 50 clinical ovarian tumors for telomerase activity
- Completed characterization of 50 clinical ovarian tumors for telomere length
- Developed technique to detect APBs in paraffin embedded sections
- Developed high density oligonucleotide microarrays
- Developed techniques to microdissect tumor tissue from frozen sections and isolated RNA.
- Developed techniques to amplify RNA from microdissected tissues for microarrays and real time PCR.

Reportable Outcomes

1. Caslini, C., Carlisle, A.J., Godwin, A.K., Broccoli, D. (2003) BRCA1 binding to telomeric complex in ALT positive cell lines. Telomeres and Telomerase, Cold Spring Harbor, NY (Oral Presentation)

2. Caslini, C., Carlisle, A.J., Godwin, A.K., Broccoli, D. (2003) BRCA1 binding to telomeric complex in ALT positive cell lines. The Seventh Annual Postdoctoral Research Conference, Philadelphia, 2002.

3. Ochs, M. and Godwin, A.K. Microarrays in cancer: research and applications. BioTechniques, 34:4-15, 2003.

4. Caslini, C., Frolova, N., Carlisle, A., Grobelny, J., Broccoli, D., Godwin, A.K. BRCA1 is a component of ALT-associated promyelocytic leukemia bodies (manuscript in preparation, 2003)

5. Caslini, C., Frolov, A., Godwin, A.K., Broccoli, D. Gene expression profiling of ALT-positive human ovarian surface epithelial cells reconstituted for telomerase activity identified a role for tankerase I (manuscript in preparation, 2003).

Conclusions

The presence of telomerase activity in the majority of tumors and the absence of activity in most human somatic cells has made telomerase an attractive target for cancer therapeutics. Telomerase inhibition can arrest the growth of tumor cells both *in vivo* and *in vitro*. Although these approaches deserve close attention, the presence of telomerase-independent mechanisms for telomere maintenance should not be ignored. Tumors using a telomerase independent mechanism, i.e., ALT (Alternative Lengthening of Telomeres), to maintain telomeric arrays would most likely be refractory to treatment with telomerase inhibitors. Likewise, the ALT pathway represents a salvage pathway that may be activated in tumors in order to overcome therapeutic effects of telomerase inhibitors. It is our hypothesis that identification of the genes that contribute to telomerase independent telomere maintenance in human cells would allow for the development of strategies to combat growth of a significant percentage of ovarian tumors and/or may suggest strategies for prevention. We have made substantial progress towards identifying gene

expression changes relevant to ALT and malignant transformation of the ovarian surface epithelial cells. Functional validation of the role of these candidates in malignant transformation of ovarian surface epithelial cells and/or ALT is ongoing. Those genes that are determined to be important in these processes will represent new targets for diagnosis and therapy.

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Appendix
Figures and Tables

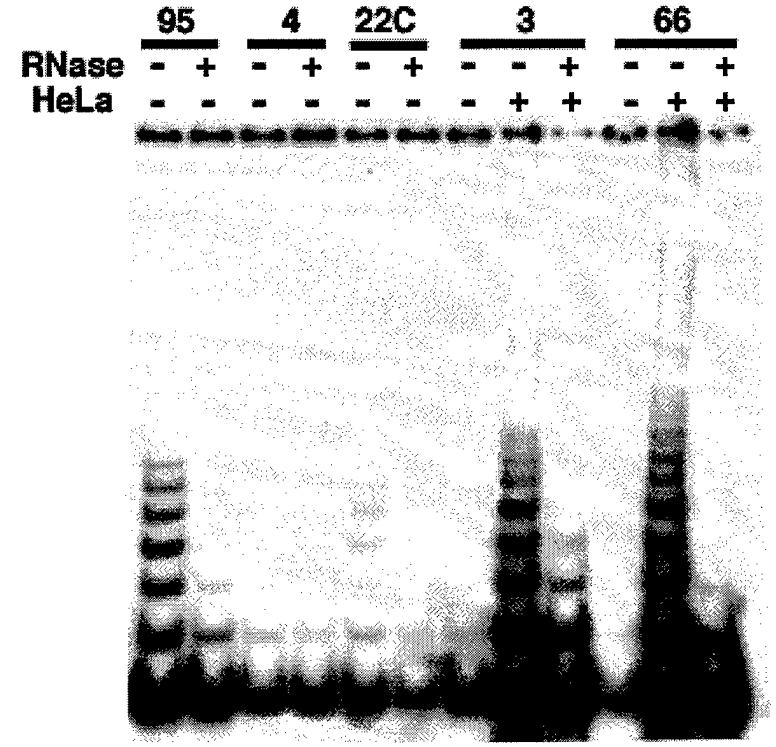

Figure 1. TRAP assay to detect telomerase activity in 5 ovarian tumors. Tumors with no detectable telomerase activity (e.g., #66) were mixed with HeLa extract to confirm that lack of telomerase activity was not due to a diffusible inhibitor. All reactions are carried in the absence (-) and presence (+) of RNase. As expected, destruction of the RNA template of telomerase inhibits the formation of reaction products.

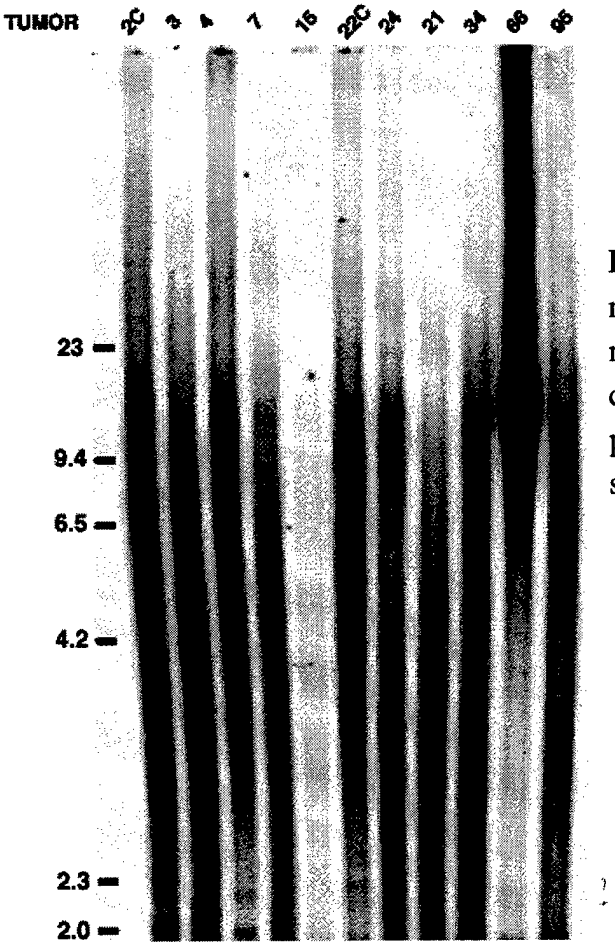

Figure 2. Southern analysis of telomere length in a number of ovarian tumors. Note that tumor #66, negative for telomerase activity (see Figure 1 above) contains ultralong telomeres characteristic of the ALT pathway. Molecular weight markers, in kilobases, are shown on the left.

Table 1. Tumors analyzed for telomerase activity. Grade and histotype are included where the information was available.

Sample	Telomerase	Grade	Histotype
UPN 1	+	III	papillary serous adenocarcinoma
UPN 2C	+	III	undifferentiated adenocarcinoma
UPN 3	+	III	endometrioid carcinoma
UPN 4	+	III	recurrent papillary serous adenocarcinoma
UPN 7	-	IV	papillary serous adenocarcinoma
UPN 9	+	III	met. serous papillary carcinoma
UPN 13	+	II	endometrioid adenocarcinoma
UPN 15	-	III	endometrioid adenocarcinoma
UPN 16	+	I	rec.mucinous adenocarcinoma
UPN18	-	III	papillary adenocarcinoma
UPN 20	-	III	papillary adenocarcinoma
UPN 22C	+	II	papillary serous cystadenocarcinoma
UPN 23	-	IV	mixed mullerian tumor of the endometrium
UPN 24	+	IV	adenocarcinoma
UPN 25	-	IV	mucinous adenocarcinoma
UPN 31	-	I	endometroid adenocarcinoma
UPN 32	-	IV	papillary adenocarcinoma
UPN 34	-	I	endometrioid adenocarcinoma
UPN 55	+	III-IV	papillary carcinoma
UPN 62	+	III	recurrent papillary serous adenocarcinoma
UPN 66	+	I	mixed Mullerian
UPN 86	+	III	mixed Mullerian
UPN 95	+	II	clear cell carcinoma
UPN 132	-	III	bil. Papillary serous cystadenocarcinoma
UPN 165	+		stromal cell tumor
UPN 167	+	II	endometrioid adenocarcinoma
UPN 229	+		mucinous cystadenoma
UPN 238	+	IV	endometrioid adenocarcinoma
UPN 248	+	III	met. serous papillary carcinoma
UPN 249	+	III	met. serous papillary adenocarcinoma
UPN 251	+		
UPN 272	+		papillary serous carcinoma
UPN 275	-		mucinous cystadenocarcinoma
UPN 282	+		
UPN 286	+		invasive papillary mucinous cystadenocarcinoma
UPN 297	+		
UPN 299	+	III	papillary serous adenocarcinoma
UPN 300	+	III	rec. endometroid adenocarcinoma
UPN 319	+		
UPN 320	-	III	mixed mesodermal tumor
UPN 323	+		invasive endometrioid adenocarcinoma
UPN 328	+		
UPN 340	+	I	endometrioid adenocarcinoma
UPN 353	-		papillary serous adenocarcinoma

UPN 356	+		papillary serous carcinoma
UPN 357	+		papillary serous adenocarcinoma
UPN 361	+		papillary serous adenocarcinoma
UPN 403	-		
UPN 533	+	III	papillary serous adenocarcinoma